

**Title: Aortic Stenosis and PhosphodiEsterase Type 5 inhibition (ASPEN)**

**NCT: NCT01275339**

**Aortic Stenosis and PhosphodiEsterase Type 5 inhibition (ASPEN)**  
Study Protocol

**Objective:** To conduct a randomized pilot study to evaluate the tolerability of chronic PDE5 inhibition (with tadalafil) and its effects on left ventricular (LV) structure and function in patients with aortic stenosis (AS).

**Clinical / Scientific Problems to be Addressed:**

- 1) Currently, AS is considered a “surgical disease” with no medical therapy available to improve any clinical outcomes.
- 2) Surgical valve replacement has a number of risks associated with it, including stroke and death. Many patients with severe aortic stenosis and symptoms—for whom surgery would be indicated—do not undergo surgery for a variety of reasons related to co-morbidities, advanced age, or other factors.
- 3) LV structural and functional abnormalities are markers of increased risk for adverse outcomes in patients with AS.
- 4) Animal models of pressure overload have demonstrated a favorable impact of PDE5 inhibition on LV structure and function, but this has not been tested in humans with AS.
- 5) Patients with AS who have diabetes mellitus (DM) have increased LV hypertrophic remodeling, worse LV function, and worse clinical outcomes than non-diabetic patients.
- 6) Adjunctive medical therapy has the potential to improve clinical outcomes in patients with AS.

**Hypotheses:**

- 1) PDE5 inhibition with tadalafil will have a favorable impact on LV structure by reducing LV mass and myocardial fibrosis.
- 2) PDE5 inhibition with tadalafil will have a favorable impact on LV function by improving measures of LV systolic and diastolic function.
- 3) Administration of tadalafil will be well tolerated in patients with AS.
- 4) Diabetes will alter the response to PDE5 inhibition in patients with AS.

**Potential Impact:** PDE5 inhibition is a novel strategy to improve clinical outcomes in patients with AS using medical therapy. This study will provide first-in-human data on the use of tadalafil in patients with AS, generating important preliminary data for designing a larger clinical trial.

**Long-Term and Short-Term Goals:**

The **long-term goal** of this research program is to develop novel medical therapies for aortic stenosis that will delay the progression of disease leading to symptoms and/or valve replacement by: 1) reversing maladaptive hypertrophic remodeling; 2) improving ventricular function; 3) reducing pulmonary artery pressure; and/or 4) halting or reversing valve stenosis. The **short-term goal** is to acquire pilot data on PDE5 inhibition that will enable the PI to design a larger clinical trial to test the hypothesis that in patients with aortic stenosis PDE5 inhibition will delay the progression of disease and improve clinical outcomes.

**Background and Rationale:**

Aortic stenosis (AS) is common and, as the population ages, increasing in prevalence. Although some estimates are higher,<sup>1,2</sup> AS is present in at least 2% of individuals > 65 years of age.<sup>3</sup> Aortic

*sclerosis*—thickening and calcification of the valve leaflets without restricted leaflet motion—is the precursor to aortic stenosis and is present in ¼ of individuals > 65 years of age. **From these and other data, a conservative estimate is that over 1 million people have aortic stenosis in the U.S and over 10 million people are likely to have its pathologic precursor, aortic sclerosis.** The only effective treatment is aortic valve replacement, which has significant morbidity and mortality associated with it. Indications for replacement of a severely stenotic valve include symptoms such as chest pain, heart failure, or syncope. At least 1/3 of patients with severe symptomatic AS do not undergo operative intervention largely due to advanced age, left ventricular dysfunction, and associated co-morbidities.<sup>4,5</sup> Once symptoms develop, the average life expectancy is < 5 years. The onset of symptoms can markedly change one's life and functional capacity.

**To date, there is no medical therapy for aortic stenosis proven to delay progression of disease, symptom onset or progression, or time to valve replacement.** The pathobiology of aortic stenosis is characterized by at least three problems: 1) ventricular dysfunction due to hypertrophic remodeling and pressure overload; 2) pulmonary hypertension; and 3) progressive valve stenosis. Although long held to be a necessary response to pressure overload, **hypertrophic remodeling of the left ventricle** may not be necessary to “compensate” for pressure overload and maintain ventricular function.<sup>6-8</sup> Several animal models have shown that ventricular function is maintained despite inhibiting the development of ventricular hypertrophy (by various means) in the setting of pressure overload.<sup>9-11</sup> Hypertrophic remodeling of the ventricle is characterized by fibrosis and myocyte/ventricular hypertrophy that leads to contractile dysfunction and increased peri-operative and long-term morbidity and mortality.<sup>12-19</sup> Diabetes adversely affects LV hypertrophic remodeling and LV function in patients with AS.<sup>20,21</sup> **Pulmonary hypertension is common in patients with aortic stenosis:** among those with severe AS, it is present in up to 65% and characterized as severe in 15-20% of patients.<sup>22,23</sup> Our own data from Washington University demonstrate that in a cohort of 1080 patients undergoing valve replacement for severe AS, pulmonary hypertension was present in ~50% (average mean pulmonary artery pressure was 24.7 mmHg in the whole cohort and 30.8 mmHg in the sub-group with pulmonary hypertension). In a higher risk subset of 29 patients with severe AS referred for a transcatheter aortic valve replacement, 66% had pulmonary hypertension (average mean pulmonary artery pressure was 30.2 mmHg in the whole cohort and 36.5 mmHg in the sub-group with pulmonary hypertension). The presence of pulmonary hypertension is associated with worse heart failure symptoms and increased peri-operative mortality.<sup>22,24,25</sup> Nonetheless, for patients with severe AS and associated severe pulmonary hypertension, surgery provides a mortality benefit while medical therapy has a dismal prognosis.<sup>24,25</sup> Finally, over the last decade it has become clear that **calcification and progressive stenosis of the valve** is not a passive “degenerative” process; there is an active biology underlying and driving these changes with similarities—but also key differences—to atherosclerosis.<sup>26</sup> Efforts to elucidate these mechanisms and attempts to alter them pharmacologically are in their infancy.

The molecular pathogenesis of AS is only beginning to be understood. One pathway that has emerged is the nitric oxide (NO)—guanosine 3',5'-cyclic monophosphate (cGMP) pathway, which affects **tissue calcification, cardiac hypertrophy, and pulmonary vasodilation**; downregulation of this pathway appears to be deleterious. NO mediates its biologic activity, in part, through generation of cGMP, which is selectively hydrolyzed by phosphodiesterase type 5 (PDE5). Recently it has been observed that inhibition of PDE5 (which reduces hydrolysis/breakdown of cGMP) blunts/reverses hypertrophic growth of the heart, reduces oxidative stress (which is associated with valve calcification), and improves pulmonary vasodilation. These observations suggest that **upregulation of NO-cGMP signaling via PDE5 inhibition may prevent disease progression in AS.** Tadalafil is a PDE5 inhibitor that has been widely used with a strong safety profile in pulmonary hypertension and erectile dysfunction.

**There is evidence to suggest that PDE5 inhibition may be helpful in preventing, halting, or reversing the hallmarks of AS pathobiology:**

1) Hypertrophic ventricular remodeling: In a pressure-overload mouse model, sildenafil (another PDE5 inhibitor) altered hypertrophic remodeling of the ventricle.<sup>27,28</sup> In this model, the aorta is surgically banded to constrict it causing an impediment to flow that increases afterload and mimics the physiology of aortic stenosis. In control animals, PDE5A contributed 35-45% of total cGMP-esterase activity; *in the pressure-overloaded mice, total cGMP-esterase activity increased 20% over controls and the component attributable to PDE5A was 60% of this total.*<sup>27</sup> **Thus, PDE5 is an abundant and viable target for inhibition in the myocardium, particularly in the setting of pressure overload.** When initiated prior to aortic banding (onset of pressure overload), sildenafil blunted the development of cardiac hypertrophy and fibrosis. When initiated after aortic banding—at a point in time when LV mass had already increased by 50% (much like patients with severe AS)—sildenafil reversed the hypertrophic remodeling process. Not only did sildenafil favorably alter ventricular structure, but it also improved ventricular function compared with control. Sildenafil had a favorable structural *and* functional impact on the murine ventricle in the setting of pressure overload.

2) Pulmonary hypertension: Based on studies in heart failure patients with secondary pulmonary hypertension, there is evidence that sildenafil causes an acute and sustained improvement in pulmonary artery pressure and exercise capacity.<sup>29-33</sup>

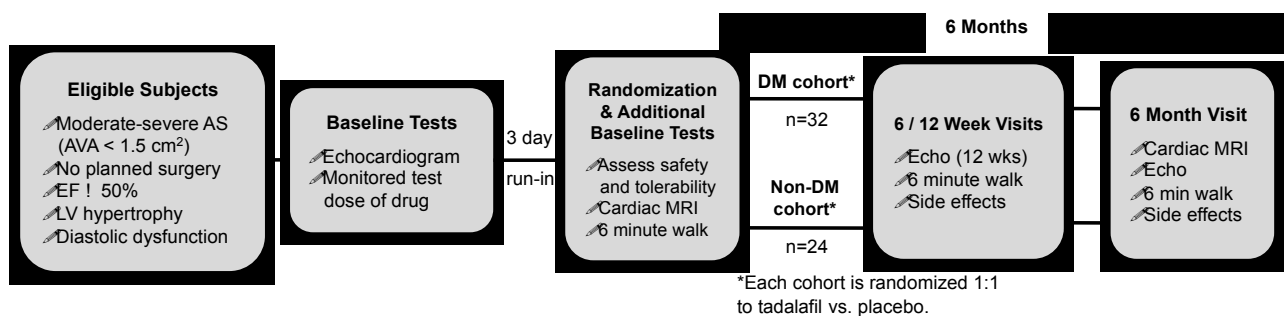
3) Valve stenosis: Although there is less data to support a potential effect of sildenafil on the valve itself, there is some data to suggest that it may reduce fibrosis and calcification in the valve, processes which are central to the progressive stenosis. Sildenafil has been shown to reduce oxidative stress and improve functional coupling of nitric oxide synthase (NOS).<sup>34</sup> Oxidative stress and uncoupling of NOS have recently been shown to be associated with calcification in human aortic valves.<sup>35-37</sup>

These preliminary studies and observations suggest that **PDE5 inhibition holds promise as a medical therapy for aortic stenosis.** There are currently three FDA-approved PDE5 inhibitors: sildenafil (Viagra, Revatio), tadalafil (Cialis, Adcirca), and vardenafil (Levitra). Both sildenafil and tadalafil are approved for the treatment of erectile dysfunction and pulmonary artery hypertension. Sildenafil was the first PDE5 inhibitor approved and, consequently, has been the most studied. With respect to their cardiac/hemodynamic effects, invasive hemodynamic studies have shown that the PDE5 inhibitors are highly similar in their acute cardiovascular effects.<sup>31</sup> Several studies have already demonstrated that sildenafil is safe and well tolerated in patients with cardiovascular disease and an ACC/AHA Expert Consensus document addresses the use of sildenafil in patients with cardiovascular disease.<sup>38</sup> Specifically, in patients with primary pulmonary hypertension or heart failure with secondary pulmonary hypertension, sildenafil and tadalafil are safe with respect to acute and long-term hemodynamics, improve pulmonary artery pressures, do not cause systemic hypotension, and improve clinically meaningful outcomes.<sup>29-33,39-41</sup> In patients with coronary artery disease, sildenafil has been shown to be safe, even having a favorable effect on coronary blood flow and measures of ischemia.<sup>42,43</sup> With respect to its effect on blood pressure, on its own tadalafil produces no significant decrease in systemic blood pressure compared to placebo. Studies in which tadalafil was combined with various antihypertensive medications (ACE-inhibitors, angiotensin receptor blockers, calcium channel blockers, and  $\beta$ blockers) showed that tadalafil either had no additional effect on blood pressure reduction or a very modest effect, reducing systolic pressure < 8 mm Hg and diastolic pressure < 4 mm Hg (data summarized in the package insert). The ACC/AHA writing group concludes: “consistent with the anticipated effects resulting from an increase in cGMP levels in vascular smooth muscle, sildenafil possesses vasodilatory properties, which result in mild, generally clinically insignificant decreases in blood pressure when taken alone.”<sup>38</sup> In patients with left-sided heart failure (like those in this study), systemic blood pressure is either unaffected or decreases minimally when measured invasively after administration of sildenafil.<sup>29,30,32</sup> The available data suggest that administering a PDE5 inhibitor to patients with aortic stenosis should be safe and well tolerated. We recently performed an invasive hemodynamic study to test the safety and acute hemodynamic effects of a single dose of a PDE5 inhibitor (sildenafil) in patients with severe symptomatic AS. We demonstrated that a single

dose of a PDE5 inhibitor is safe and well-tolerated in patients with severe AS and is associated with acute improvements in pulmonary and systemic hemodynamics resulting in biventricular unloading.<sup>44</sup> These findings support the need for longer-term studies to evaluate the role of PDE5 inhibition as adjunctive medical therapy in patients with AS.

Available evidence suggests that PDE5 inhibition is a promising strategy for improving clinical outcomes in patients with AS. PDE5 inhibition may exhibit a favorable effect via altered hemodynamics and/or a direct effect on tissue remodeling. In order to design a larger clinical trial to test efficacy, pilot data is needed on short-term tolerability and the effects of PDE5 inhibition on hemodynamics, LV structure/function, and relevant clinical metrics (quality of life, 6 minute walk).

### **Study Design and Intervention Plan:**



Subjects will be recruited from cardiology and cardiac surgery colleagues at Washington University School of Medicine. The research team will screen the clinics (via Allscripts) and the echocardiography lab to identify eligible subjects. Permission to approach potential subjects will be obtained from one of the subject's treating physicians.

Participants must be willing to attend all scheduled visits, take the study drug as directed and have the required tests and procedures done for each visit. They will be asked to fast for eight hours before certain study visits. Once consent is obtained, baseline testing will be arranged and performed, including a fasting blood draw, physical exam (vitals, height, weight, waist circumference), quality of life, 6 minute walk, and an echocardiogram. The Baseline Study Visit will include observation and monitoring of a dose of tadalafil (20mg oral). Heart rate, blood pressure, and O<sub>2</sub> saturation will be measured for at least 90 minutes after the dose of drug is given (the peak hemodynamic effect of the drug has been shown to be 90 minutes). If, during this 90 minute monitoring period, there is a time when 3 successive systolic blood pressures average < 100 mmHg with or without symptoms (lightheadedness, syncope, or related symptoms), a subject will not be included in any further aspects of the study. If the systolic blood pressure remains > 100 mmHg, the subject will be given an initial prescription for tadalafil (20mg orally once per day for 3 days). A heart rate / blood pressure (HR/BP) monitor will be given to enrolled subjects. They will be instructed as to how to use it and given instructions to keep a daily diary where they will record the time the study drug is taken and the time/reading of the HR/BP machine; side effects can also be recorded on the diary.

A Randomization Study Visit will be arranged for ~5-9 days later (allowing for flexibility in scheduling). Prior to this visit, all subjects will have taken tadalafil for 3 days, followed by 2-6 days off the drug. Heart rate, blood pressure, and O<sub>2</sub> saturation will be measured; the daily diary will be reviewed. If the drug has been well-tolerated for the 3 day run-in phase, the subject will be randomized to tadalafil vs. placebo (1:1 randomization). Subjects with diabetes will be in the diabetes cohort and those without diabetes will be in the non-diabetes cohort. Reasons to exclude from randomization would include: 1) systolic blood pressure < 100 mmHg; 2) not tolerating the medication (either due to an intolerable side effect or some other reason); or 3) no longer willing to comply with the requirements of the study. Randomization will be carried out by an investigational pharmacist.

The subject and PI/study coordinator will be blinded to whether the subject has been assigned tadalafil or placebo. An MRI with and without gadolinium will be performed on all randomized subjects prior to initiation of the study drug. Upon randomization, subjects will take 20 mg (once daily) of the study drug for 3 days before having the dose increased to 40 mg (once daily). Instructions will again be given to maintain a daily diary of dose timing, HR/BP, and side effects. If the increase to 40mg daily is not tolerated (either due to a side effect or systolic blood pressure < 100mmHg), then the dose will be decreased back to 20mg daily.

Follow-up study visits will occur at 6 and 12 weeks and 6 months after randomization. Testing will occur at each of those visits, including blood draws, HR/BP, quality of life, 6 minute walk, echocardiogram (12 weeks and 6 months), and MRI (6 months). The MRIs will be performed in the Center for Clinical Imaging Research (CCIR). Other testing will occur in the Clinical Trials Unit (CTU) on the 11<sup>th</sup> floor of the Center for Advanced Medicine (CAM).

If a subject develops low pressure (systolic blood pressure < 100 mmHg) with or without symptoms of lightheadedness, presyncope, or syncope, the study drug will be discontinued for 48 hours. The PI will discuss the findings with one of the subject's treating physicians. If the subject's treating physician is agreeable, the study drug will be re-initiated 48 hours later (1 dose missed), perhaps after an alteration has been made to his/her other cardiac medications (i.e. discontinuation of another anti-hypertensive and/or a decrease in the dose of another cardiac medication).

Subjects will be contacted by phone weekly for the first 4 weeks of the study and then monthly thereafter to inquire about the HR/BP log, any side effects, or other questions/problems.

*Removal of subjects from the study:* If at any time during the study a subject would like to discontinue participation, he/she will be removed from the study and no further follow-up testing or monitoring will occur. If a subject needs to discontinue the study drug before completion of the study protocol, the PI will determine whether to obtain further testing (MRI, echo, 6 minute walk, blood, etc.) at the time the study drug is discontinued; the decision as to whether to obtain further testing will largely depend on how long the subject has been taking the study drug. The PI may withdraw any subject whose health or well-being may be threatened by continuation in this study. The following instances require termination of subjects: 1) subjects who have adverse events or intercurrent conditions that require discontinuation of study medication; 2) subjects who, in the opinion of the investigator, should be discontinued for their well being; 3) subjects who are unable to understand task instructions or to perform tests adequately; or 4) subjects who fail to comply with protocol requirements.

### ***Clinical Variables:***

<b>Baseline Clinical Data</b>	<ul style="list-style-type: none"> <li>• Age</li> <li>• Gender</li> <li>• BSA</li> <li>• BMI</li> <li>• Waist circumference</li> <li>• Heart rate</li> <li>• O<sub>2</sub> saturation</li> <li>• Systolic BP</li> <li>• Diastolic BP</li> <li>• Smoking history</li> <li>• History of HTN</li> <li>• Peripheral Vascular Disease</li> <li>• CAD               <ul style="list-style-type: none"> <li>○ Detailed coronary anatomy if available</li> </ul> </li> <li>• Prior MI</li> </ul>
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	<ul style="list-style-type: none"> <li>• Metabolic Syndrome</li> <li>• Diabetes mellitus <ul style="list-style-type: none"> <li>◦ Microvascular complications</li> </ul> </li> <li>• GFR</li> <li>• NYHA class</li> <li>• Syncope</li> <li>• Angina</li> <li>• Medications <ul style="list-style-type: none"> <li>◦ ACE-I or ARB</li> <li>◦ <math>\beta</math>-blockers</li> <li>◦ Statins</li> <li>◦ CCB</li> <li>◦ Aldosterone antagonist</li> <li>◦ Insulin</li> <li>◦ Oral diabetic meds</li> </ul> </li> <li>• KCCQ</li> <li>• 6 minute walk</li> <li>• Review for presence and severity of common side effects with PDE5 inhibitors</li> </ul>
<b>Randomization Visit</b>	<ul style="list-style-type: none"> <li>• Heart rate</li> <li>• O<sub>2</sub> saturation</li> <li>• Systolic BP</li> <li>• Diastolic BP</li> <li>• <b><i>Review for presence and severity of common side effects with PDE5 inhibitors</i></b></li> <li>• <b><i>Exclusions from being randomized:</i></b> <ul style="list-style-type: none"> <li>- No longer willing to comply with the requirements of the study</li> <li>- Systolic blood pressure &lt; 100 mmHg</li> <li>- Not tolerating the medication (either due to side effect or some other reason)</li> </ul> </li> </ul>
<b>Study Visits</b>  <b>6 Weeks</b> <b>12 Weeks</b> <b>6 Months</b>	<ul style="list-style-type: none"> <li>• Heart rate</li> <li>• O<sub>2</sub> saturation</li> <li>• Systolic BP</li> <li>• Diastolic BP</li> <li>• KCCQ</li> <li>• 6 minute walk</li> <li>• Review for presence and severity of common side effects with PDE5 inhibitors</li> </ul>
<b>6 Minute Walk</b>	<ul style="list-style-type: none"> <li>• Administered by research coordinator at the time of study visits</li> </ul>
<b>Kansas City Cardiomyopathy Questionnaire (KCCQ)</b>	<ul style="list-style-type: none"> <li>• Self-administered, 23-item questionnaire that quantifies physical limitations, symptoms, self-efficacy, social interference and quality of life</li> </ul>
<b>Side Effects</b>	<ul style="list-style-type: none"> <li>- Headache</li> <li>- Flushing</li> <li>- Dyspepsia</li> <li>- Nasal congestion</li> <li>- Runny nose</li> <li>- Respiratory tract infection</li> <li>- Back pain</li> <li>- Extremity pain</li> <li>- Nausea</li> <li>- Myalgia</li> <li>- Sudden decrease or loss of vision in one or both eyes</li> </ul>

	<ul style="list-style-type: none"> <li>- Priapism</li> <li>- Lightheadedness</li> <li>- Presyncope</li> <li>- Syncope</li> <li>- Decrease or loss of hearing</li> </ul>
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**Blood Collection:**

<b>Baseline</b>	<ul style="list-style-type: none"> <li>• <b>Standard</b> <ul style="list-style-type: none"> <li>- CMP (including fasting blood glucose)</li> <li>- CBC</li> <li>- FLP</li> <li>- HbA1c (for diabetics)</li> <li>- BNP</li> </ul> </li> <li>• <b>Research Analyses</b> <ul style="list-style-type: none"> <li>- 3 tubes to plasma and buffy coat aliquots</li> <li>- 1 tube for whole blood</li> <li>- 1 tube for serum aliquots</li> </ul> </li> </ul>
<b>Follow-up Study Visits</b>	<ul style="list-style-type: none"> <li>• <b>Standard</b> <ul style="list-style-type: none"> <li>- BNP</li> </ul> </li> <li>• <b>Research Analyses</b> <ul style="list-style-type: none"> <li>- 2 tubes for plasma aliquots</li> <li>- 1 tube for serum aliquots</li> </ul> </li> </ul>

**Echocardiogram Measurements:**

	<ul style="list-style-type: none"> <li>• LVESD, LVEDD, septal and posterior wall thickness (res up on ventricle for accurate measurement)</li> <li>• Ejection fraction (EF)</li> <li>• Magnified view of 4Ch and 2Ch for accurate LV volumes</li> <li>• AS severity: LVOT<sub>d</sub> (use same value for all echoes analyzed in study), AVA, AVA<sub>index</sub>, gradients (mean and peak)</li> <li>• Stroke volume and cardiac output: from LVOT<sub>VTI</sub>, LVOT<sub>d</sub>, and HR</li> <li>• Severity of MR and AR</li> <li>• Mitral valve inflow (PW Doppler): <i>load modulation views</i></li> <li>• Tissue Doppler (septal and lateral annulus)</li> <li>• Longitudinal strain: 3 apical views</li> <li>• LV twist: 3 parasternal short-axis views</li> <li>• Pulmonary vein (PW Doppler)</li> <li>• <b>RV assessment:</b> <ul style="list-style-type: none"> <li>- TAPSE (M-mode of tricuspid annulus)</li> <li>- Tei (get TV inflow – PW at leaflet tips)</li> <li>- Tissue Doppler (PW) of the TV annulus</li> <li>- Speckle tracking view of RV</li> </ul> </li> <li>• <b>Pulmonary artery pressure estimate:</b> <ul style="list-style-type: none"> <li>- PASP estimate from tricuspid regurgitation (TR) jet (with saline if needed) and subcostal view of IVC</li> <li>- PADP estimate from the PR jet</li> <li>- PA mean from the above and from the RV outflow acceleration time</li> <li>- RVOT PW for acceleration time and VTI</li> </ul> </li> </ul>
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**MRI Measurements:**

	<ul style="list-style-type: none"> <li>• Performed with and without contrast</li> </ul>
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	<ul style="list-style-type: none"> <li>• Measurements on chamber dimensions made with Argus software</li> <li>• Ejection fraction (EF)</li> <li>• LV volumes (LVESV and LVEDV)</li> <li>• LV mass</li> <li>• Fibrosis: <ul style="list-style-type: none"> <li>- Pre-contrast myocardial T<sub>1</sub> times (12 segments individually and global LV)</li> <li>- Post-contrast myocardial T<sub>1</sub> times (12 segments individually and global LV)</li> <li>- Late gadolinium enhancement (both # of segments with LGE and volume/mass of LGE)</li> </ul> </li> <li>• 3D multiparametric systolic strain analysis (regional and global strain)</li> </ul>
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**Study Visits:**

	Monitored dose of drug	Blood	Echo	6 min walk	KCCQ	MRI
Baseline Visit	X	X	X	X	X	
Randomization Visit	Vitals and side effects					X
6 Weeks	Vitals and side effects	X		X	X	
12 Weeks	Vitals and side effects	X	X	X	X	
6 Months	Vitals and side effects	X	X	X	X	X

**Study Design Summary:**

<b>Patient Population</b>	<ul style="list-style-type: none"> <li>• Patients with moderate to severe aortic stenosis with increased LV mass in whom valve replacement is not planned.</li> </ul>
<b>Study Design</b>	<ul style="list-style-type: none"> <li>• Single center, randomized, double-blind, placebo controlled study</li> </ul>
<b>Study Phase</b>	<ul style="list-style-type: none"> <li>• 4</li> </ul>
<b>Drug</b>	<ul style="list-style-type: none"> <li>• Tadalafil (Adcirca) 20 mg tablets (commercial)</li> </ul>
<b>Sample Size</b>	<ul style="list-style-type: none"> <li>• This study will include 56 subjects randomized to placebo or drug</li> <li>• There will be 2 cohorts of patients based on diabetic status</li> <li>• The diabetes cohort will include 32 subjects randomized to placebo or drug</li> <li>• The non-diabetes cohort will include 24 subjects randomized to placebo or drug</li> <li>• To allow for the possibility of drop-out of subjects after consent is signed but before randomization occurs (mostly due to the potential for not tolerating the test dose of the drug or the run-in phase), we will plan to enroll up to 75 subjects in the study</li> </ul>
<b>Intervention</b>	<ul style="list-style-type: none"> <li>• <b>Run-in phase:</b> <ul style="list-style-type: none"> <li>- All subjects receive tadalafil 20mg daily</li> </ul> </li> <li>• <b>Randomization (1:1 – tadalafil:placebo) within each cohort:</b> <ul style="list-style-type: none"> <li>- Placebo</li> <li>- Drug: tadalafil 40mg daily (if tolerated) or resume taking 20mg</li> </ul> </li> </ul>
<b>Inclusion Criteria</b>	<ol style="list-style-type: none"> <li>1) Moderate to severe aortic stenosis (AVA &lt;1.5 cm<sup>2</sup>)</li> <li>2) LV hypertrophy as evidenced by LV mass index &gt;95 g/m<sup>2</sup> for women and &gt;115 g/m<sup>2</sup> for men</li> <li>3) Diastolic dysfunction as evidenced by tissue Doppler e' (average of septal and lateral) ≤ 7 cm/s</li> <li>4) None or minimal symptoms related to aortic stenosis (NYHA ≤ 2)</li> <li>5) The subject and treating physician are not planning on a valve replacement</li> </ol>

	<p>procedure to occur during the next 6 months.</p> <ol style="list-style-type: none"> <li>6) EF <math>\geq</math> 50%</li> <li>7) Ambulatory</li> <li>8) Normal sinus rhythm</li> <li>9) 18 years of age and older</li> <li>10) Able and willing to comply with all the requirements for the study</li> </ol>
<b>Exclusion Criteria</b>	<ol style="list-style-type: none"> <li>1) Need for ongoing nitrate medications</li> <li>2) SBP &lt; 110mmHg or MAP &lt; 75mmHg</li> <li>3) Moderately severe or severe mitral regurgitation (3 or 4+ MR)</li> <li>4) Moderately severe or severe aortic regurgitation (3 or 4+ AR)</li> <li>5) Contraindication to MRI or unable to tolerate MRI</li> <li>6) Creatinine clearance &lt; 30 mL/min</li> <li>7) Cirrhosis</li> <li>8) Pulmonary fibrosis</li> <li>9) Increased risk of priapism</li> <li>10) Retinal or optic nerve problems or unexplained visual disturbance</li> <li>11) If a subject requires ongoing use of an alpha antagonist typically used for benign prostatic hyperplasia (BPH) (prazosin, terazosin, doxazosin, or tamsulosin), SBP &lt; 120 mmHg or MAP &lt; 80 mmHg is excluded</li> <li>12) Need for ongoing use of a potent CYP3A inhibitor or inducer (ritonavir, ketoconazole, itraconazole, rifampin)</li> <li>13) Current or recent (<math>\leq</math> 30 days) acute coronary syndrome</li> <li>14) O<sub>2</sub> sat &lt; 90% on room air</li> <li>15) Females that are pregnant or believe they may be pregnant</li> <li>16) Any condition which the PI determines will place the subject at increased risk or is likely to yield unreliable data</li> <li>17) Unwilling to provide informed consent</li> </ol>
<b>Endpoints</b>	<p><b>Primary Endpoints:</b></p> <ol style="list-style-type: none"> <li>1) Change in diastolic function as measured by tissue Doppler e' on echo from baseline to 6 months</li> <li>2) Change in LV longitudinal peak systolic strain by echo from baseline to 6 months</li> <li>3) Change in LV Mass as measured by MRI from baseline to 6 months</li> </ol> <p><b>Secondary Endpoints:</b></p> <ol style="list-style-type: none"> <li>1) Change in other echocardiographic indices of diastolic function, including E/e' and deceleration time</li> <li>2) Change in other echocardiographic indices of systolic function, including stroke volume, LV twist, and stress-corrected midwall shortening and whether these changes are influenced by diabetes, EF, CAD, and LV hypertrophic remodeling</li> <li>3) Safety and tolerability – frequency of the following: hypotension (SBP &lt; 90 mmHg), symptomatic hypotension (symptoms of presyncope or syncope associated with SBP &lt; 90), syncope, hospitalization for a cardiac reason, myocardial infarction, new onset or worsening heart failure, and new sustained arrhythmia requiring intervention)</li> <li>4) Change in LV hypertrophic remodeling (relative wall thickness, LV chamber dimensions, and wall thickness)</li> <li>5) Change in echocardiographic indices of diastolic function (stiffness, viscoelasticity, and a load independent index of diastolic filling) using a novel methodology (PDF formalism)</li> <li>6) Change in myocardial fibrosis (ECV) as assessed by MRI</li> </ol>

- 7) Change in 3D multiparametric strain and ejection fraction by MRI
- 8) Change in 6 minute walk
- 9) Change in quality of life (KCCQ)
- 10) Change in systemic markers of fibrosis and oxidative stress
- 11) Change in pulmonary artery pressure
- 12) Change in pulmonary vascular resistance
- 13) Change in systemic blood pressure
- 14) Change in RV function, including TAPSE, S', and Tei index
- 15) Change in AS severity (AVA, mean and peak transvalvular gradients) and whether there are sub-groups that progress faster/slower)
- 16) Change in BNP

#### **Pre-specified secondary analyses:**

- 1) Evaluate co-variables that may influence the response of the primary endpoints to therapy (DM, AS severity, CAD, SBP, SAC, valvuloarterial impedance, fibrosis, baseline LV mass, baseline  $e'$ , baseline LV systolic strain)
- 2) What baseline variables and/or changes over the study period correlate with any improvements in KCCQ and/or 6 minute walk.
- 3) Evaluate the primary and secondary endpoints in the pre-specified cohorts of diabetic and non-diabetic subjects. Within cohort and between cohort differences will be analyzed to evaluate the relationship between diabetes status and response to PDE5 inhibition.
- 4) Evaluate how biomarkers of collagen turnover, fibrosis, oxidative stress, and inflammation change over time with PDE5 inhibition and evaluate whether baseline biomarker levels (or change in biomarker levels) can predict (or correlate with) the changes in cardiac structure and/or function with PDE5 inhibition.
- 5) Use a novel method (PDF formalism) for assessing the diastolic properties and function of the LV (eg. stiffness, viscoelasticity, load independent index of filling, etc.); evaluate whether (and which of) these indices change with PDE5 inhibition and whether these changes are influenced by diabetes, EF, CAD, and LV hypertrophic remodeling.
- 6) Evaluate whether PDE5 inhibition is associated with change in myocardial fibrosis as assessed by extracellular volume (ECV) (by MRI  $T_1$  mapping) and whether this change is influenced by diabetes, baseline ECV, and baseline biomarkers of fibrosis.

#### **Analysis of Peripheral Blood:**

- The following signaling pathways relevant to the pathobiology and aortic stenosis will be studied:
  - Hypertrophic remodeling
  - Calcification
  - Fibrosis
  - Inflammation
  - Oxidative stress
  - Nitric oxide – cyclic GMP
- The types of analyses to be performed include:
  - Quantity of RNA, protein, oxidized lipids, and other systemic markers
  - Genetic mutations and polymorphisms
  - GWAS
- **GWAS Study collaboration:** For the GWAS component of this study, we will

	collaborate with investigators at the University of Miami (PI: David Seo, MD; IRB #20080787; “Genetics of Aortic Valve Disease”). This is an ongoing project, funded by the University of Miami. We will send blood specimens to them for genotyping. These specimens will have unique barcode labels and not have identifying information on the tubes. Additionally we will send some demographic/clinical data relevant to the genetic analyses, but without any identifiers. <i>This collaboration has already been IRB approved under HRPO 09-1026 (PI: Lindman).</i>
<b>Data Analysis</b>	<ul style="list-style-type: none"> <li>Statistical analyses for the primary and secondary endpoints will be conducted with a mixed model repeated measures analysis of variance of the relevant parameter at baseline and 6 months, testing the interaction between group (tadalafil vs. placebo) and time. This is equivalent to testing whether the baseline to study endpoint change in one group is different than the change in the other and uses all available information. A <math>p &lt; 0.05</math> will be considered significant. The primary analysis of the trial will be a pooled analysis, including subjects from both cohorts comparing the tadalafil and placebo treated subjects. Secondly, within each cohort, endpoints will be compared between tadalafil and placebo treated subjects.</li> </ul>
<b>Interim Analyses</b>	<ul style="list-style-type: none"> <li>2 interim analyses are planned to assess safety and efficacy as well as conditional power; the details of these planned interim analyses are spelled out below under the Data and Safety Monitoring section</li> </ul>
<b>Power Calculations</b>	<ul style="list-style-type: none"> <li>This is a pilot study. There is no data on effect sizes and variance of change for these endpoints in this patient population resulting from PDE5 inhibition. Even if this study generates statistically significant results, follow-up larger studies will be needed to more definitively determine efficacy. The main purposes of this pilot study are to assess safety and tolerability and determine effect sizes and variance for several important, clinically relevant endpoints that will enable future studies to be appropriately planned and powered.</li> <li>Assuming a standard deviation of change in <math>e'</math> of 2.2 cm/s, the power to detect a 2 cm/s difference between drug and placebo for a sample size of 56 (28 receiving drug, 28 receiving placebo) is 91%. The power calculation is based on evaluating the difference in change from baseline to 6 months via a two sample t-test (2-tailed) with type I error (alpha) set to 5%. This is similar to the change observed in comparable populations receiving comparable treatments.</li> <li>Assuming a standard deviation for the change in <b>LV mass</b> of 25 grams, a sample size of 56 (28 receiving drug, 28 receiving placebo) will be able to detect a difference in LV mass change from baseline to 6 months of 23 grams when evaluated with a two sample t-test (2-tailed) that is powered at 92% with a type I error (alpha) equal to 5%. This is a standardized effect size of 0.92. Assuming the mean baseline LV mass is 240 grams, this would reflect a difference of 9.5% in the change in LV mass between the 2 groups, which is conservative compared to prior studies on regression of LV mass after valve replacement in those with AS.</li> <li>For the diabetes cohort, we will have 80% power to detect a standardized effect size of 1.04 for change in each of the primary endpoints. This is comparable (and even conservative) to the change observed in <math>e'</math> and LV longitudinal systolic strain in patients with metabolic syndrome treated with an aldosterone antagonist.</li> </ul>
<b>Target Study Start Date</b>	<ul style="list-style-type: none"> <li>September 2012</li> </ul>
<b>Target Study Completion Date</b>	<ul style="list-style-type: none"> <li>December 2015</li> </ul>
<b>Compensation</b>	<ul style="list-style-type: none"> <li>Total compensation for completion of the entire protocol = \$450 <ul style="list-style-type: none"> <li>Completion of Baseline Testing: \$75</li> <li>Completion of Safety and Tolerability Visit: \$75</li> </ul> </li> </ul>

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|  | <ul style="list-style-type: none"> <li>- Completion of 6 Week Study Visit: \$50</li> <li>- Completion of 12 Week Study Visit: \$125</li> <li>- Completion of 6 Month Study Visit: \$125</li> </ul> |
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### **Data and Safety Monitoring Plan:**

Individuals involved in data and safety monitoring for this study:

- **Brian R. Lindman, MD** – Principal Investigator
- **Alan C. Braverman, MD** – Professor of Medicine (Cardiology) (independent monitor)
  - **Expertise:** Dr. Braverman is a non-invasive cardiologist who sees patients with a variety of cardiac disorders and specializes in caring for patients with a bicuspid aortic valve and genetically triggered aortic diseases. He also has experience participating in clinical trials involving drug interventions. He has a keen understanding of the issues relevant to carrying out this study in a manner that protects human subjects.
- **Lewis Chase, MD** – Professor of Medicine (Endocrinology and Metabolism) and Chair, Scientific Advisory Committee for the Washington University ICTS (independent monitor)
  - **Expertise:** Dr. Chase has extensive experience in leading and supervising clinical and basic research. He will provide a non-cardiologist perspective for the oversight of this study. As chair of the scientific advisory committee of the ICTS, he is well qualified for this task.

The principal investigator will take primary responsibility for following enrolled patients for any adverse events and will discuss situations in which the patient's participation in this study may have led to an adverse event with Drs. Braverman and Chase. Drs. Braverman, Chase and the PI will review the data on enrolled subjects every 6 months. If a participant has a serious adverse event the case will be reviewed within 7 days to see if any modifications to the protocol need to be made. This review will occur before enrolling another subject to see if any modifications to the protocol need to be made.

### **Safety:**

#### Adverse Events:

An adverse event (AE) is any untoward medical occurrence in a subject administered a pharmaceutical product, regardless of causality assessment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a study drug, whether or not considered related to the study drug.

An abnormality identified during a medical test (e.g., laboratory parameter, vital sign, ECG data, physical exam) should be defined as an AE only if the abnormality meets one of the following criteria:

- Induces clinical signs or symptoms;
- Requires active intervention;
- Requires interruption or discontinuation of study drug;
- The abnormality or investigational value is clinically significant in the opinion of the Investigator.

#### Serious Adverse Events:

A serious adverse event (SAE) is defined as an adverse event, occurring at any dose that meets any of the following criteria:

- Results in death,
- Is life threatening: that is, poses an immediate risk of death as the event occurred,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in a persistent or significant disability or incapacity, or
- Is a congenital anomaly/birth defect in the offspring of the subject (whether the subject is male or female).

The recording of AEs will start after the subject has signed the informed consent form and will end at the 6 month follow-up visit. The PI will monitor each subject closely for AEs and the PI will record all **observed** or **volunteered** AEs.

Among other adverse events, each study participant will have the following information tracked:

- Development of symptomatic hypotension
- Syncope
- Hospitalization for a cardiac reason
- New onset or worsening heart failure
- Sustained arrhythmia requiring intervention
- Myocardial infarction

We will review the occurrence of any of the above on each patient in the study and determine whether these events are believed to be due to the patient's participation in the study or simply a known and anticipated clinical event related to the subject's aortic stenosis and/or other cardiac diseases. All serious adverse events (SAEs) deemed to be related or possibly related to participation in the research will be reported to the HRPO within 24 hours, or at the time of continuing review (for all other adverse events) using the myIRB Notification System.

### **Interim Analyses:**

- Two interim analyses are planned:
  - 1) After 24 total subjects (diabetic and non-diabetic) are enrolled
  - 2) After 16 diabetic subjects are enrolled

At each of these interim analyses, an unblinded analysis will be performed by Ken Schechtman, PhD. The purpose of the interim analysis will be to assess safety and tolerability and conditional power. The study will not be stopped early for efficacy because the main purpose of this study is to determine effect sizes and standard deviation of change for several surrogate endpoints and to acquire data on safety and tolerability of PDE5 inhibition in this patient population. The study may be stopped early for safety reasons. The conditional power of the study will be determined based on the actual effect sizes (change in the primary endpoints from baseline to 6 months) and variance (standard deviation of the change in the primary endpoints from baseline to 6 months) of the enrolled subjects in each arm (treatment and placebo). If based on the effect size and SD of change from the enrolled subjects the conditional power is less than originally planned (90% for the pooled analysis and 80% for the diabetic cohort), the total sample size and/or the sample size of the diabetic cohort may be increased to improve the power to detect a difference in one or more of the primary endpoints. A decision to increase the sample size will consider whether such an increase would be feasible in terms of funding and additional time for subject recruitment. The following are guidelines for the interim analysis for the statistician and DSMB. The unblinded analyses will only be seen by the statistician and DSMB.

***Conditional Power Calculation for the Primary Endpoints – Performed at the Interim Analysis:***

- The conditional power will be calculated using an unmatched two-tailed t-test comparing treatment vs. placebo groups with the actual effect sizes (change in  $e'$ , change in longitudinal systolic strain, and change in LV mass from baseline to 6 months) and actual variance of those measures for the enrolled subjects
- The conditional power will be characterized as favorable, promising, or unfavorable and the following actions are suggested for the DSMB. The primary characterization of the conditional power will be based on the primary endpoint demonstrating the greatest power.
  - **Favorable:**  $\geq 90\%$  for pooled analysis,  $\geq 80\%$  for diabetic cohort
    - The study is adequately powered and should continue enrollment to the originally planned sample size
  - **Promising:**  $50\% \leq \text{conditional power} < 80\%$  (diabetic cohort) or  $90\%$  (pooled)
    - Based on the effect sizes and variance from the enrolled subjects, the conditional power of the study is “promising” but somewhat less than originally planned. The sample size may be increased to whichever of the following yields a lower sample size:
      - The sample size will be increased so that the conditional power is increased to what was originally planned (80% for the diabetic cohort, 90% for the pooled analysis)
      - The cap for increasing the sample size is 40 for the diabetic cohort and/or 64 for the total cohort
      - If the conditional power cannot be increased to 80% by the increase in sample size to the pre-specified cap, then an increase in the sample size will likely not be recommended
  - **Unfavorable:** conditional power  $< 50\%$ 
    - The study will continue enrollment to the originally planned sample size to gather important information of effect sizes and variance for several primary and secondary endpoints that will be used to plan future studies

**Privacy and Data Integrity:**

Every 6 months, we will review the process by which consents are obtained and stored and how data is entered into the database. We will discuss any known breaches of privacy or data integrity to implement procedures that will prevent further occurrences.

**Data Monitoring Reports:**

Every 6 months, the PI will take responsibility for generating a data monitoring report based on the above criteria and discussions. Any recommendations for changes in the protocol or implementation of the study (if needed) will be included in this report.

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